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THE INHIBITIVE EFFECT OF OX-BILE ON B. TYPHOSUS

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Bile media have been used extensively, as recommended by Jackson,[1] for rapid isolation of B. coli in sanitary water analysis. They have also been proposed for the isolation of B. typhosus from the blood of patients suffering with typhoid fever (Conradi,[2] Kayser,[3] Fornet[4] and others). That the action of bile on B. coli, however, is inhibitive has been conclusively shown by Jordan[5] and confirmed by Obst.[6]

Since common air and water bacteria and intestinal organisms are for the greater part eliminated through the use of bile mediums, such mediums have certain advantages in the isolation of B. coli and B. typhosus. Bile has even been regarded by some as favoring the growth of these species. Lately, however, experiment tends to show that the effect is inhibitory rather than favorable on typhoid as well as on colon bacilli.

REVIEW OF THE LITERATURE

As far back as 1892, Corrado[7] stated that bile has but a slight effect on the growth of typhoid bacilli; in some instances, the reaction of the bile might have been even slightly inhibitive as stated by that author.

That bile may stimulate the growth of typhoid bacilli was asserted by Fischer,[8] Babes,[9] and Neufeld,[10] while Leubuscher,[11] Talma,[12] and Braun,[13] reported a transitory inhibition. Pies[14] maintains that ox-bile inhibits B. typhosus to a

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[1] Biological Studies by the Pupils of William Thompson Sedgwick, Boston, 1906.

[2] Deutsch. med. Wchnschr., 1906, 32, p. 58.

[3] München. med. Wchnschr., 1906, 53, pp. 823, 896, and 1953.

[4] Ibid., 1906, 53, p. 1862.

[5] Jour. Infect. Dis., 1913, 12, p. 326.

[6] Jour. of Bacteriol., 1916, 1, p. 73.

[7] Centralbl. f. Bakteriol., 1892, 12, p. 696.

[8] Ueber die Wirkung der Galle auf Typhus und Milzbrandbazillen, Inaug. Diss., Bonn, 1894.

[9] Berl. klin. Wchnschr., 1899, 17, p. 362.

[10] Ztschr. f. Hyg., 1900, 34, p. 454.

[11] Ztschr. f. klin. Med., 1890, 17, p. 472.

[12] Centralbl. f. Bakteriol., I, 1901, 29, p. 367.

[13] Arch. d. Sci. Biol., 1901, 8, p. 158.

[14] Ueber das Wachstum von Typhus und Colibazillen in Galle, Inaug. Diss., Strassburg, 1907.

more extensive degree than it does B. coli. In his investigations he found one specimen of human bile that inhibited both organisms markedly. According to the same author, the addition of nutrient substances to the bile accelerated the growth of the bacilli, thus neutralizing the inhibitory action of the bile. Dunschmann,[15] who studied the effect of bile and bile salts on B. typhosus and B. coli, reported a favorable action of the bile on B. typhosus and slight inhibition of the growth of B. coli. Hirokawa[16] found that B. coli, B. typhosus and B. paratyphosus A and B develop very well in bile mediums. The author mentions one case of distinct inhibition of typhoid bacilli and states that the power of restraining is slightly or not at all impaired through heat. Fränkel and Krause[17] have reported that human bile stimulates the growth of typhoid bacilli, B. coli, Staphylococcus pyogenes, and Vibrio cholerae. Fornet[18] was able to find but slight difference in the inhibitive effect of steam heated and fresh bile on B. typhosus. When a few typhoid organisms are inoculated into bile, they suffer inhibition to a relatively greater degree according to Fornet. Lange and Roos[19] found that rabbit's bile inhibited typhoid bacilli only to a very slight degree. Tonney, Caldwell and Griffin[20] concluded that "The effect of lactose peptone bile enrichment when used in the attempt to isolate bacilli from urine and feces is that of inhibition of the growth of the bacilli. As a rule, typhoid organisms that are demonstrable by direct plating are lost by passage through lactose bile." Nichols[21] states that rabbit bile in vitro may be antiseptic to B. typhosus, B. paratyphosus A, B. coli and B. dysenteriae. The cholera vibrio which is known to grow best in alkaline culture media, grew well in fresh alkaline bile. The antiseptic action is largely due to its alkalinity. It is apparently possible to protect the rabbit to some degree against gall-bladder infection by a previous injection of sodium carbonate.

From this general survey of the literature it is plain that the results obtained by different workers are more or less at variance.

STRAINS OF TYPHOID BACILLI USED

In our experiments the strains employed were obtained as follows:

1. B. typhosus 1 from the laboratory stock cultures. Source unknown.

2. B. typhosus 2, from Dr. Theobald Smith's laboratory, was isolated on Aug. 28, 1900, from the spleen of a typhoid patient.

3. B. typhosus B was isolated on Dec. 17, 1914, from the urine of a typhoid patient.

4. B. typhosus J was isolated from the blood of a patient on Dec. 15, 1914.

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[15] Ann. de l'Inst. Pasteur, 1909, 23, p. 29.

[16] Centralbl. f. Bakteriol., I, O., 1909-1910, 53, p. 12.

[17] Ztschr. f. Hyg., 1899, 32, p. 91.

[18] Arch. f. Hyg., 1907, 60, p. 134.

[19] Arb. a. d. k. Gsndhtsamte, 1915, 57, p. 5.

[20] Jour. Infect. Dis., 1916, 48, p. 239.

[21] Jour. Exper. Med., 1915, 24, p. 497.

5. B. typhosus C was isolated from the stool of a patient on Jan. 16, 1915.

All the suspensions used were made up from broth cultures incubated at 37 C. for 24 hours. In all of the experiments the colony count was made after 48 hours at 37 C.

EXPERIMENTS 1, 2, AND 3

As the addition of other nutrient substances may have a more or less beneficial influence on the different strains, the following mediums were made up in the first 3 series of experiments:

A. Full fresh ox-bile, 1000 c.c., agar, 15 gm.
B. Full fresh ox-bile, 1000 c.c., agar 15 gm., peptone (Difco), 10 gm.
C. Full fresh ox-bile, 1000 c.c., agar, 15 gm., peptone (Difco), 10 gm., and lactose, 10 gm.
D. Full fresh ox-bile, 1000 c.c., agar, 15 gm., peptone (Difco), 10 gm., and glycerol, 10 gm.

The agar, peptone and lactose were dissolved in the bile without addition of water as rapidly as possible by gentle boiling. The medium was filtered and allowed to cool and again boiled and refiltered without titration. It was then tubed and sterilized in the autoclave for 3 minutes at 15 lbs. pressure. The elimination of the precipitate as much as possible during the cooling and refiltering is of great importance for a later count of the colonies. Table 1 shows the inhibitive action of bile on the growth of typhoid bacilli. The better growth obtained in the glycerol series may be attributed to a lessening of the bactericidal effect of the bile or to the favoring of the organisms by the glycerol. Typhoid bacilli are thus restrained through the use of whole fresh ox-bile.

TABLE 1 (UNDILUTED BILE)

AVERAGE RESULTS OBTAINED WITH PLAIN BILE AND BILE CONTAINING DIFFERENT NUTRIENT SUBSTANCES COMPARED WITH THOSE OBTAINED WITH PLAIN AGAR

Strains	Plain Agar	A Bile Agar	B Peptone Bile Agar	C Lactose Peptone Bile Agar	D Glycerol-Peptone Bile Agar
No. 1	224	153	121	70	128
No. 2	122	0	0	1	101
B	290	82	44	89	150
J	175	92	98	124	102
C	209	13	13	20	98

In this series, as recorded in Table 2, 50% bile (diluted with distilled water) was used and the mediums prepared in the same manner as in the first experiment. The comparative results set forth seem to indicate that a great number of the organisms refuse to grow on bile mediums. The glycerol-peptone-bile-agar had a slight inhibitive effect on some of the strains. The colonies on the glycerol-containing medium were much larger and showed a strikingly vigorous growth.

TABLE 2 (50% BILE)

AVERAGE RESULTS OBTAINED WITH THE SAME NUTRIENT SUBSTANCES BUT WITH A DIFFERENT DILUTION OF BILE

Strains	Plain Agar Control	Bile Agar	Peptone Bile Agar	Lactose Peptone Bile Agar	Glycerol Peptone Bile Agar
No. 1	192	120	50	60	200
No. 2	150	73	15	6	60
B	441	73	53	175	400
J	800	500	300	550	430
C	157	7	16	11	63

TABLE 3 (10% BILE)

RESULTS OBTAINED WHEN NUTRIENT SUBSTANCES WERE USED WITH A HIGHLY DILUTED BILIARY SOLUTION

Strains	Plain Agar Control	Bile Agar	Peptone Bile Agar	Lactose Peptone Bile Agar	Glycerol(10%) Pentone Bile Agar
No. 1	600	83	18	582	0
No. 2	212	45	96	187	10
B	543	50	0	470	3
C	386	13	327	300	8

In Exper. 3, 10% bile (diluted with distilled water) was prepared with agar. peptone, lactose and glycerol as in Exper. 1. The results given in this 3rd table are of interest not only because the bile retained its suppressing power in such a high dilution, but also because the inhibitive effect of lactose-peptone-bile-agar here is practically eliminated while the addition of 10% glycerol brings about a uniform inhibition of the cells of all 4 strains. These findings indicate that bile inhibits a large proportion of the otherwise viable cells and the more concentrated the fluid, the greater its effect.

STERILIZATION OF BILE BY FILTRATION

Freshly obtained bile was filtered through a Buchner funnel and passed through a Nordmeyer bougie of 185 by 25 mm., tested for sterility and divided with aseptic precautions in portions of 10 c.c. in test tubes and kept on ice. At this temperature the bile often turns cloudy with the formation of a brown precipitate in the bottom of the tubes, which readily disappears when the fluid is brought to room temperature.

EXPERIMENT 4

The strains were plated with 5 c.c. of the filtered bile which was heated to 45 C. and mixed with 5 c.c. of agar prepared by dissolving 15 gm. of agar in 500 c.c. of tap water. The results were compared with plain agar.

EXPERIMENT 5

A suitable suspension of typhoid bacilli was mixed with plain agar to which 1 c.c. of filtered sterile bile was added after the agar was cooled down to 43 C. Control series were made in plain agar without bile. Again the inhibitive effect is plainly apparent here, but very much weaker. In this case nutrient agar plays an important rôle in furnishing the more nutrient substances favoring the growth of the bacilli.

TABLE 4

Showing the Inhibitive Effect of Unheated Filtered Bile

Strains	Plain Agar	Filtered Bile Agar
No. 1	755	7
No. 2	406	3
J	409	10
C	59	7

On this medium B. typhosus is practically unable to grow.

TABLE 5

Showing the Inhibitive Effect of Filtered Bile

Strains	Plain Agar	Bile Agar
No. 1	28	15
No. 2	46	23
B	40	39
J	130	130
C	53	32

Experiment 6

A loopful of a 24-hour typhoid suspension was added to 10 c.c. of broth and from this suspension after thorough mixing one loop was carefully plated out by washing the loop in 1 c.c. of 0.85% salt solution previously added to the plate. Several plates were made so as to obtain a fair average of the total number of organisms carried by the loop. After this the same procedure was followed except that 10 c.c. of the sterile filtered bile replaced the broth. Table 6 gives the numbers of bacteria surviving exposure to filtered ox-bile for different periods of time. A marked decrease of the otherwise viable cells takes place after the tubes have been shaken. Within the first 5 minutes a great majority are destroyed or their growth prevented. After 20 minutes the increase from the suviving organisms is fairly constant.

TABLE 6

Showing the Rapid Inhibition of Otherwise Viable Cells

Strains	Controls	Minutes						
		1	5	10	15	20	30	40
No. 1	25	18	31	39	20	20	18	28
No. 2	15	1	3	4	4	1	2	3
B	57	35	20	32	24	23	35	34
J	52	2	1	2	5	5	13	17
C	45	22	29	32	35	25	35	40

Experiment 7

The same experiment was repeated with one strain; the bile used was unheated, filtered in 1 instance, and heated, filtered in the other (3 minutes at 15 lbs. in the autoclave). The results in this experiment indicate that the inhibitive power of filtered bile, when heated for 3 minutes in the autoclave at 15 lbs., is not destroyed, thus confirming the findings of Fornet and Neufeld. There is a marked inhibition of the organisms after 10 minutes in the nonheated medium, but this may indicate a slighter reaction on the part of the nonheated bile when compared with that of the heated.

TABLE 7

SHOWING THE EFFECTS OF HEATED AND NONHEATED BILE*

	Minutes						
	1	5	10	15	20	30	40
Heated filtered	108	100	112	100	100	98	69
Nonheated filtered..	124	110	50	95	125	118	194

* Strain C: 208 organisms carried by the loop used.

EXPERIMENT 8

In Table 8 the results are given from platings with a suitable suspension of typhoid bacilli in plain agar and the same agar with varying amounts of precipitated mixed bile salts. The mediums were sterilized for 3 minutes in the autoclave at 15 lbs. pressure. A pronounced reduction of the organisms takes place when bile salts are added to nutrient agar. This reduction is constant.

TABLE 8

SHOWING THE INHIBITIVE EFFECT OF BILE SALTS

Strains	Plain Agar Controls	0.05 Per Cent.	Bile-Salts-Agar		
No. 1	387	312	178	241	161
No. 2	920	332	250	226	170
B	1,020	876	900	626	480
J	23	20	16	11	0
C	960	870	840	636	354

SUMMARY

1. The recorded observations show that a great number of the viable cells of B. typhosus are affected through the use of bile media, and further multiplication prevented. Bile has a distinct inhibitive effect on the growth of typhoid bacilli.

2. This inhibition can be reduced to a certain extent by the addition of glycerol.

3. There is no marked difference between the inhibitory power of bile sterilized by heat and that of bile sterilized by filtration through a Nordmeyer bougie.

4. Bile salts distinctly retard the growth of typhoid bacilli.